Food Sci. Biotechnol. 22(1): 79-85 (2013)
DOI 10.1007/s10068-013-0011-2

RESEARCH ARTICLE

Anti-atherogenic Properties of Emulsified Perilla Oil (EPO) in Apo E KO Mice and Plasma Lipid Lowering Effects of Rice Porridge Containing EPO in Healthy Young Adults

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Received: 5 June 2012 / Revised: 19 August 2012 / Accepted: 18 September 2012 / Published Online: 28 February 2013

Abstract The objective of this study was to develop a rice porridge containing perilla oil rich in α-linolenic acid which demonstrated anti-atherogenic effect in the previous study. Lipid lowering effect of emulsified perilla oil (EPO) was examined in apo E KO mice (n=18) and that for rice porridge containing EPO (RPEPO) was investigated in the pilot scale human study (n=20). Inhibitory effects of EPO on the plasma lipid levels and fatty streak lesion size in apo E KO mice were similar to those observed in mice fed perilla oil (PO), suggesting that EPO is applicable form of PO in manufacturing rice porridge product with PO's health benefit properties. In human study with young adults aged between 20-35 years old, changes in plasma triglyceride concentration of subjects consumed RPEPO for 30 days as a breakfast was significantly lowered than those for subjects consumed rice porridge (p<0.05), of which calorie levels were the same (333 kcal/meal).

Keywords: perilla porridge, apo E KO mice, young adult, plasma lipid, fatty streak lesion size

Introduction

Perilla oil produced from perilla seeds (*Perilla frutescens*) is used as cooking oil in Asian countries. The major fatty acid of perilla oil is α-linolenic acid (ALA), *n*-3 polyunsaturated fatty acid, of which concentration is over 60% of the total fatty acids (1). In animal studies, perilla oil demonstrated plasma cholesterol-lowering (2) and hypolipidemic effects (3). Moreover, perilla oil rich in ALA exerted preventive effect on atherosclerosis (4), chemically induced cancer (5), and also showed beneficial effects on the improvement of immune (6) and mental functions (7). Although perilla oil is highly unsaturated, radical scavenging activities against DPPH, superoxide, and hydroxyl radical were greater compared to other edible oils such as soybean oil, corn oil, safflower oil, etc, since it is usually used as unrefined oil (8). Health benefits of perilla oil are partially explained by high content of ALA in it, which is a known precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the body. Eicosanoids synthesized from long chain polyunsaturated fatty acid (PUFAs), EPA, and DHA, are effective on the prevention of atherosclerosis. Besides these, perilla oil seems to have other functions on lipid metabolism via regulating the expression of related transcription factors such as hepatic sterol regulatory element-binding protein (SREBP) and peroxisome proliferator-activated receptor α (PPARα) (9). Moreover, it also reveals anti-inflammatory effects via suppressing oxidative stress in aorta, liver, brain, and kidney (9,10). Skip a meal or grab a convenient food is common behavior who wants to save their time. The prevalence is particularly high among students and working populations between 20-30 years old. Convenient foods are commercially prepared food designed for ease of consumption. Fresh-cut product, ready meal (ready-to-eat) products, and instant food product are the top 3 categories of convenient foods, world widely (11). In Korea, markets for the ready meals such as soup, sundae, *sunsik* (partially cooked grain), noodle, porridge, etc (12) are also expanding rapidly, revealing 6.1% growth rate in 2011, which was the highest among the processed food items

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(13). Among ready meal products, rice porridge is one of steady growing items. Sea foods, vegetables, nuts, seeds, beans, etc are ingredients traditionally used in the preparation of rice porridge (14). Recently, ingredient such as sericulture (15), lotus root (16), and green laver (17) are used to increase the functional properties of the rice porridge, which are known to have anti-hypertension, cholesterol lowering activity, improvement of cognitive recognition, anti-inflammatory effect, anti-diabetic, and anti-tumor activity (15-17). However, the expected health benefits of the functional rice porridges have yet been reported in human. Therefore, in this study we would like to develop a functional rice porridge containing perilla oil, of which importance are cited as plasma lipid lowering activity as well as retardation of fatty streak formation in aortic sinus in animal study (8). In Korea where the working population is rapidly increased and westernized fast foods prevails widely, deteriorated disease such as type 2 diabetes mellitus, cancer, and cardiovascular disease are likely to become important public health issues. For the study, lipid lowering effects of emulsified perilla oil was first studied in animal to verify its functional equivalence to perilla oil. And then, lipid lowering effect of rice porridge product containing emulsified perilla oil was investigated in a pilot scale human study with young adults.

Materials and Methods

Fats and porridge samples Lard, perilla oil, and emulsified perilla oil (EPO) for the animal study, and rice porridge (RP) and rice porridge containing EPO (RPEPO) for the human study were provided by N company (Seoul, Korea). The major fatty acids of perilla oil used in this study are α-linolenic acid, linoleic acid, and oleic acid of which concentration are reported as 61.1, 14.3, and 13.2% of total fatty acids, respectively (1). Recipes for RP and RPEPO are shown in Table 1. The total calorie for porridges per meal is the same, 333 kcal/meal.

Preparation of EPO The process of EPO preparation was as follows; Oligosaccharide solution was prepared by mixing with water. Pentaglycerolmyristic acid was dissolved in the oligosaccharide solution at 60-70°C. Perilla oil (20 mL/min) was added slowly (20 mL/min) to the pentaglycerolmyristic acid mixture solution using homomixer at 5,000 rpm. Final mixture was further homogenized for 30 min at 6,000 psi. The compositions for EPO were perilla oil 30%, pentaglycerolmyristic acid 3.75%, oligosaccharide 48.25%, and water 18%.

Animals and diets Male apolipoprotein E Knockout (apo E KO, n=18) mice of 6 weeks was purchased from SLC Inc., (Hamamatsu, Japan). Each animal was kept in an individual cage under the standardized conditions with a 12 h light-dark cycle, a temperature (22±1°C), and humidity (55±5%) during the entire experiment. After 1 week of acclimatization, each mouse was randomly assigned into 3 groups as lard group (LD), perilla oil group (PO), and emulsified perilla oil group (EPO) according to the fat sources. Atherogenic diet was prepared as shown in Table 2 and provided every day. Food consumption and body weight of mouse were measured weekly.

Table 1. Recipe for rice porridge used in the experiment (g)

Ingredient	Types of porridge[1)]	
	RPEPO	RP
Soaked rice	13.40	39.52
Soaked glutinous rice	13.40	43.68
Perilla seed flour	26.90	-
Perilla oil	2.70	-
Peanut flour	13.40	5.42
Salt	1.10	0.80
Sugar	0.40	-
Water	228.60	410.58
Total	300.00	500.00
Calorie (kcal)	333.00	333.00

[1)]RPEPO, rice porridge containing emulsified perilla oil; RP, rice porridge

Plasma collection and analysis of parameters in the plasma After 12 h of fasting, mice were anesthetized by intraperitoneal administration of 30 mg/kg of Zoletil (Virbac Laboratories, Carros, France) and 10 mg/kg of xylazine (Rompun; Bayer Korea, Seoul, Korea). Blood from the inferior vena cava was immediately centrifuged to obtain the plasma. Plasma was stored at −80°C for the further analysis.

Triglyceride (AM157S-K; Asan Pham., Seoul, Korea), total cholesterol (AM202-K; Asan Pham.), and high density lipoproteincholesterol (AM203-K; Asan Pham.) were determined using commercially available kits. The concentration of LDL-cholesterol was calculated. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also assayed using a commercially available kit (AM101-K; Asan Pharm.).

Assessment for fatty streak lesion size on aortic sinus The upper section of the heart including 2 mm ascending aorta was cut and fixed it in 4% buffered formalin over night at 4°C. It was rinsed with 30% sucrose solution and rinsed with phosphate buffered saline (PSB) (10 M, pH 7.2). It was embedded in the tissue freezing medium (Tissue-Tek OCT compound; Miles Inc., Elkhart, IN, USA). From each sample, 5-μm thickness section was cut

Table 2. Composition of the experimental diet (%)

Ingredient	Experiment diet[1)]		
	LD	PO	EPO
Casein	6.45	6.45	6.45
Soy protein	11.17	11.17	11.17
DL-Methionine	0.26	0.26	0.26
Corn starch	36.53	36.53	36.53
Sucrose	4.56	4.56	4.56
Cellulose	7.74	7.74	7.74
Oligosaccharide	13.82	13.82	13.82
Lard	8.60	0	0
Perilla oil	0	8.60	8.60
Cocoa butter	3.44	3.44	3.44
Coconut oil	1.72	1.72	1.72
Mineral mix[2)]	3.01	3.01	3.01
Vitamin mix[2)]	0.86	0.86	0.86
Choline bitartrate	0.17	0.17	0.17
Cholesterol	1.25	1.25	1.25
Sodium cholic acid	0.43	0.43	0.43

[1)]LD, high cholesterol (1.25%) diet containing lard; PO, high cholesterol (1.25%) diet containing perilla oil; EPO, high cholesterol (1.25%) diet containing emulsified perilla oil
[2)]AIN-76 (Dyets Inc., Bethlahem, PA, USA)

using a cryostat followed by mounting it on the coated slide glass. The distal portion of the aortic sinus is recognized by the 3 valve cusps which are the junctions of the aorta to the heart. Slides shown 3 valve cusps were stained with Oil Red O for the neutral lipid and counterstained with hematoxylin. Lipid accumulation was photographed (40×, Olympus CH30; Olympus, Tokyo, Japan) and the lesion size was measured using a computer-assisted light microscopy with DMC advance image software (Techsan., Ltd., Suwon, Korea).

Subjects and a pilot scale human study design Twenty people aged between 20-35 years olds were recruited from the university through poster advertising and email campaigns. This study followed human study protocols of university hospital of P. National University. Subjects participated in this study were explained the purpose of experiment and clinical procedures of drawing blood, of which usage will be limited to plasma lipid analysis only. Subjects were also told to have a free will to drop this study anytime without any disadvantages of doing it. And also benefits and rewards for participation the study were individually explained. Subjects agreed with our human study objects were signed the written consent before the experiment began. After a screening interview, subjects who are non-allergic to perilla oil were selected. For the study, 20 volunteers were assigned into 2 groups randomly in the order - a RP and RPEPO intake group. Subjects were asked to consume the porridge as a breakfast for 30 days, and to remain on their usual diet and maintain their usual physical activities during the entire experiment. Three days food records were received every week to examine the nutrient intake of subjects. Volunteers are instructed to have kimchi or vegetables in limited amount as a side dish with the porridge, if they needed.

Blood draw and body composition measurement Blood sample were drawn twice from overnight-fasted subjects. Fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) were determined automatically (Modular Analytics, Roche, Mannheim, Germany). ALT and AST were assayed using a commercially available kit as mentioned in the section of animal study.

Weight, height, and body composition were measured after 2 h fasting using Inbody (X-Scan Plus II; Jawon Medical, Guri, Korea) before and after study. Body mass index (BMI, kg/m^2), body fat amount (kg), body fat percentage (%), fat free mass (kg), skeletal muscle weight (kg), and waist/hip ratio were also calculated.

Sensory evaluation Sensory evaluations for RP, RPEPO, and black sesame porridge (Daesang FNF, Seoul, Korea) were carried out with 20 persons who participated in the human study. Samples were provided in a white bowl with same shape and size. Appearance, flavor, taste, viscosity, and overall acceptability were evaluated on 9-point scale. Point 1 is the most dislike and 9 is the most preferred.

Statistical analysis Statistical analyses were performed using SPSS package 18.0 (SPSS Inc., Chicago, IL, USA). All values were presented as mean±standard deviation (SD). Mean values of the animal study were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test as post hoc analysis to determine the significance. Data from the human study, paired *t*-test was applied to analyze the differences between pre and post test in the same group. And student's *t*-test was used to compare the delta changes between 2 groups. The results of the sensory evaluation were analyzed by ANOVA followed by Tukey's HSD. Data were considered to be significant at $p<0.05$.

Results and Discussion

Body weight and food intake in apo E KO mice Body weight changes among the experimental groups were not significantly different. Food intakes (g/day) among 3 groups were not significantly different as well (data not shown).

Table 3. Plasma lipid profiles of apo E KO mice fed high cholesterol diet containing different fats for 10 weeks (mg/dL)

Group[1)]	TG	TC	LDL-C	HDL-C
LD	109.91±15.56[a2)]	1,782.51±338.94[a]	1,757.87±336.18[a]	2.66±1.48[b]
PO	84.13±13.76[b]	1,398.79±201.23[b]	1,374.16±199.08[b]	7.81±2.37[a]
EPO	84.81±18.42[b]	1,424.52±194.08[b]	1,403.33±196.57[b]	4.23±1.09[b]

[1)]Mice group fed high cholesterol diet containing lard (LD), perilla oil (PO), or emulsified perilla oil (EPO) for 10 weeks.
[2)]Data are mean±SD (n=6 each group); [a,b]Data with different letters in the column are significantly different with ANOVA followed by Duncan's multiple range test at $p<0.05$.

Changes in plasma lipid concentrations and other parameters in apo E KO mice As shown in Table 3, plasma TG, TC, and LDL-C concentrations for the PO and EPO groups were significantly lower than those for LD group ($p<0.05$) while no differences were found in HDL-C concentration among groups. TG concentrations of PO and EPO group were decreased by 23.46 and 22.84%, respectively compared to that of LD group. TC and LDL-C concentrations of PO and EPO group were reduced by 21.53, 20.08, and 21.83, 20.17%, respectively, compared with LD group indicating that lipid lowering effects of EPO are comparable to those of PO. These results are in line with several citations demonstrated plasma lipid lowering effect of perilla oil (18-20). The possible mechanisms of perilla oil on decreasing plasma lipids concentrations might be due to suppression of hepatic lipogenic enzyme activities (9), especially fatty acid synthase (FAS) activity (18). Moreover, mitochondrial and peroxisomal fatty acid oxidation was elevated (21) by perilla oil administration. Lipogenic enzyme activities in the liver responsible for the synthesis of TC and TG are regulated by hepatic SREBP and PPARα genes. In our previous study, SREBP-1 expression was down regulated and that for PPARα was increased in perilla oil fed group thereby lipogenic enzyme activities were declined (9). Once hepatic TC and TG synthesis are inhibited, which will subsequently lead to drop plasma TC and TG level (9,18).

According to the present results, lipid lowering effects of EPO were comparable to those for PO, suggesting that EPO is applicable form of PO in manufacturing rice porridge product with PO's health benefit properties.

Fatty streak lesion size in apo E KO mice Representative images of aortic sinus are shown in Fig 1. Fatty streak lesion size in the LD, PO, and EPO group were 377,361 ±102,086, 191,255±40,244, and 203,725±50,753 μm^2, respectively. The aorta lesion size in apo E KO mice fed PO or EPO were reduced by 49.32 or 46.01%, respectively, compared with LD group, which are in accordance with our previous result (8). Plasma lipid lowering effects of PO and EPO could be one of the possible explanations for contributing this phenomenon. From this result, the beneficial effect of EPO on lipid lowering activity was

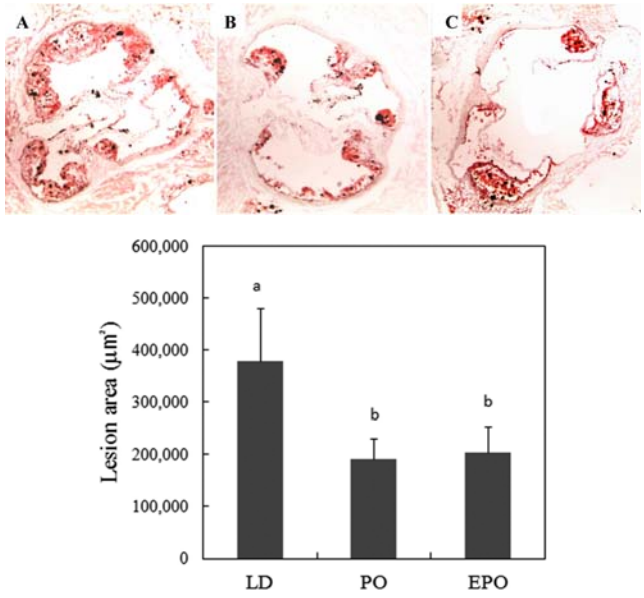

Fig. 1. Inhibitory effect of PO and EPO on the formation of fatty streak in aortic sinus of apo E KO mice fed high cholesterol diet containing different fats for 10 weeks. (A) lard (LD), (B) perilla oil (PO), (C) emulsified perilla oil (EPO) group; [a,b]Data with different letters are significantly different with one-way ANOVA followed by Duncan's multiple range test at $p<0.05$.

confirmed although related mechanism studies are yet carried out. In our previous study, the expression of ICAM-1 and VCAM-1 on endothelium in perilla oil fed apo E KO mice were decreased (8). It is well known that monocyte recruitment to the endothelium is enhanced by adhesion molecules on the surface of endothelial cells, including vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and E-selectin, which subsequently enhance atherogenesis (22).

Anthropometric data, ALT, AST of subjects Anthropometric data of subjects in 2 groups were not different in terms of mean age (25.40±3.02 vs. 25.40±2.31 years old), height (163.42±3.85 vs. 161.28±3.57 cm) and weight (58.32±5.10 vs. 54.04±5.72 kg). BMI, body fat mass, fat free mass, skeletal muscle weight of subjects after 30 days of human study were not significantly changed (Table 4). According to the anthropometric data, volunteers are

Table 4. Food intake and body composition of the subjects before and after human study for consuming different rice porridge for 30 days[1)]

	RPEPO		RP	
	Baseline	Post-test	Baseline	Post-test
Calorie (kcal)	2,178.88±316.51[2)]	2,074.53±211.12[NS]	1,887.35±396.20	1,971.24±181.83[NS]
BMI (kg/m^2)	21.85±1.70	21.67±1.81	20.77±2.08	21.03±2.27
Body fat amount (kg)	15.60±2.97	15.86±3.10	14.00±3.10	14.53±3.64
Body fat percentage (%)	26.55±2.97	27.13±3.15	25.66±3.22	26.25±3.88
Fat free mass (kg)	42.72±2.43	42.09±2.35	40.04±2.97	40.14±3.08
Skeletal muscle weight (kg)	18.63±2.95	17.06±2.74	15.95±3.76	15.85±3.27
Waist/Hip ratio	0.75±0.32	0.76±0.03	0.75±0.40	0.76±0.04

[1)]Subjects consumed rice porridge containing emulsified perilla oil (RPEPO) or rice porridge (RP) for 30 days as a breakfast; Amounts of RPEPO and RP intake for each meal were 300 and 500 g, respectively.
[2)]Mean±SD (n=10 each group); [NS]Data in the row are not significantly different (p<0.05).

Table 5. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities of subjects before and after human study for consuming different rice porridge for 30 days (Karmen unit/mL)

Group[1)]	AST		ALT	
	Baseline	Post-test	Baseline	Post-test
RPEPO	26.90±4.58[2)]	26.90±2.96[NS]	11.80±3.05	15.50±1.72[NS]
RP	27.30±4.08	26.40±5.00	10.60±4.62	15.80±3.19

[1)]RPEPO, rice porridge containing emulsified perilla oil; RP, rice porridge
[2)]Mean±SD (n=10 each group); [NS]Data in the row are not significantly different (p<0.05).

followed given instructions well and practiced. ALT and AST activities of subjects in both groups are in normal range (Table 5).

Fasting blood glucose (FBG) level of subjects FBG concentration between pre and post test in both RPEPO and RP group were increased by 3.78 (4.79%) and 6.67 mg/dL (9.36%), respectively, however, the changes in each group were not significantly different with their baseline data (Table 6). Although FBG rise are observed from both groups, the rise in RPEPO group were smaller than that of RP. This result is not in line with the one observed in the animal studies shown FBG lowering effect of perilla oil supplemented diet (8,23) or fish oil supplemented diet (24). One of possible explanation for this FBG rise might be due to the experimental assignment to the subject to have a regular breakfast with designated porridge for 30 days of human study periods. Skip a meal is prevalent in young generation, especially the breakfast (25). In this study, changes of FBS level in 2 groups were not significantly higher than baseline.

Plasma lipid profiles of subjects The plasma lipid concentrations of post-test were higher than base-line data regardless of porridge type, of which phenomenon was observed in FBG data. However, when the changes of lipid concentration between pre and post test were compared, triglyceride concentration change in RPEPO group was significantly lower than that in RP group (p<0.05, Table 6). The LDL-C and HDL-C level between pre and post test in both RPEPO and RP group were not significantly different with the base line. Subsequently change in LDL-C concentration for RPEPO which was slightly higher than RP was not significantly different. Also there was no significance in changes of HDL-C concentrations between 2 groups. No significant differences were observed in the rest of plasma lipids parameters.

According to the results, perilla oil in RPEPO seems to have effect on suppressing TG increase after rice porridge consumption even the amount of lipid in RPEPO is higher than RP (16 vs. 10%). TG lowering effects of perilla oil diet are extensively studied and we already discussed about these early in the section of the animal study in this paper.

Macronutrient composition and sensory evaluation of porridge The ratio for macronutrient, carbohydrate: protein:lipid in RPEPO used in this study is 61:23:16 that is relatively well balanced (26) except for protein which is slightly high. Most of porridge products commercially available in the market are high in carbohydrate. For example, rice porridge (86:6:5), adzuki bean porridge (77:19:4), pumpkin porridge (88:9:3), abalone porridge (76:17:7), and black sesame porridge (68:14:19) (27). Among commercial products, macronutrient composition of the black sesame porridge is proper. Sensory evaluation results are shown in Table 7. Among 3 porridges including rice

Table 6. Changes in fasting blood glucose (FBG) level and plasma lipid profiles of the subjects before and after human study for consuming different rice porridge for 30 days (mg/dL)

Variables	Group[1)]	Baseline	Post-test	Change
FBG	RPEPO	77.30±6.57[2)]	81.00±4.69	3.78±5.04[NS]
	RP	76.90±5.63	84.10±6.57	6.67±5.31
TG	RPEPO	45.63±18.28	58.63±15.45	13.00±10.82[a]
	RP	61.00±13.35	93.00±15.28	32.00±20.72[b]
TC	RPEPO	180.22±22.00	183.00±18.12	2.78±13.93[NS]
	RP	169.11±21.60	174.11±30.38	5.00±12.03
LDL-C	RPEPO	99.78±29.85	106.22±27.39	6.44±9.54[NS]
	RP	95.11±20.90	100.44±26.87	5.33±9.11
HDL-C	RPEPO	72.67±11.87	73.89±11.21	1.22±7.20[NS]
	RP	62.67±9.58	64.22±7.60	1.55±6.69

[1)]RPEPO, rice porridge containing emulsified perilla oil; RP, rice porridge; BSP, rice porridge containing black sesame
[2)]Mean±SD (n=10 each group); [NS]Data in the column are not significantly different; [a,b]Data of change with different letters between 2 groups in each lipid profile are significantly different by student t-test at $p<0.05$.

Table 7. Sensory evaluation results of various porridges

Type of porridge[1)]	Appearance	Flavor	Taste	Viscosity	Overall preference
RPEPO	5.80±1.90[NS2)]	6.15±1.50[a]	6.20±1.61[a]	4.60±1.64[b]	6.20±1.65[a]
RP	5.20±1.20	5.20±1.05[b]	5.65±1.22[a]	6.25±1.11[a]	5.80±1.15[a]
BSP	3.90±2.10	4.10±1.71[b]	4.30±2.22[b]	4.35±1.57[b]	4.45±2.01[b]

[1)]RPEPO, rice porridge containing emulsified perilla oil; RP, rice porridge; BSP, rice porridge containing black sesame
[2)]Mean±SD (n=20); [NS]Data in the column are not significantly different; [a,b]Data with different letters in the column are significantly different with one-way ANOVA followed by Tukey's HSD at $p<0.05$.

porridge containing black sesame (Daesang FNF, Seoul, Korea) of which macronutrient composition is balanced, RPEPO was highly evaluated than the others in flavor, taste, and overall preference.

In conclusion, rice porridge containing perilla oil, practically as a form of emulsified perilla oil, demonstrated plasma triglyceride lowering effect in a pilot scale human study with young adults. Take the results from the animal study together into consideration; emulsified perilla oil is suitable form of perilla oil for manufacturing rice porridge with PO's health benefits.

Acknowledgments This study was supported by the research grant (#109130-3) from the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, which is gratefully appreciated.

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